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Investigation of the Presence and Levels of Aflatoxin M1 in Labneh Cheese from Different Producers in Afyonkarahisar Using ELISA

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ABSTRACT

Aflatoxins are toxic compounds produced by *Aspergillus* species and are among the most dangerous mycotoxins. They can cause serious health effects such as cancer, birth defects, and genetic damage. Humans are mainly exposed through contaminated food and animal products like milk, dairy, and eggs. AFM1 is the metabolite of AFB1 found in milk and dairy products resulting from the consumption of contaminated feed containing AFB1 by dairy animals. In this study conducted for this purpose, the presence and level of AFM1 in labneh cheeses of different brands were investigated. In this context, 40 labneh cheese samples sold in markets were collected, and the presence of AFM1 was investigated using the ELISA method. AFM1 level was detected between 0.026-0.033 µg/kg in 10% (4/40) of the labneh cheese samples analysed. All of the samples were found below the Turkish Food Codex Contaminants Regulations limit (0.050 µg/kg). In conclusion, it is recommended to raise awareness about the potential health risks associated with the consumption of milk and dairy products contaminated with AFM1, which is an important group among Aflatoxins that harbour many health risks that may affect the general public, and to implement measures to prevent contamination in the food supply chain.

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INTRODUCTION

The term "mycotoxin," derived from the Greek word "mykes" (fungus) and the Latin "toxicum" (poison), refers to toxic secondary metabolites produced by certain fungal species (Öksüztepe and Erkan, 2016; Türel and Calapoglu, 2017). Mycotoxins can develop in a variety of foods and feeds due to the increased moisture content and temperature of the environment and can lead to toxin production by fungi. Among the significant types of mycotoxins, aflatoxins are primarily produced by Aspergillus flavus and Aspergillus parasiticus molds (Dinçel et al., 2012). Depending on exposure and dose, aflatoxins can exhibit acute or chronic effects, causing teratogenic, mutagenic, toxigenic, and carcinogenic impacts on both humans and animals (Erdoğan, 2004; Fazekas and Tarakovacs, 2005; Sengun et al., 2008). Within the food chain, humans and animals can ingest aflatoxins through contaminated food and feed. Particularly, humans are exposed to aflatoxins via milk, dairy products, and eggs derived from animals fed aflatoxin-contaminated feed (Dinçel, 2012; Akgül, 2021).

Aflatoxins are named based on their fluorescence under ultraviolet (UV) light. AFB1 and AFB2, produced by A. flavus, emit blue fluorescence, while AFB1, AFB2 (blue), AFG1, and AFG2 (green) are produced by A. parasiticus. Aflatoxin M1 (AFM1), also known as the "milk toxin," exhibits a blue-violet fluorescence and is found in milk and dairy products derived from animals fed AFB1-contaminated feed (Kocasarı Şahindokuyucu, 2014; Akgül, 2021). After ingestion of AFB1-contaminated feed, AFB1 is hydroxylated in the liver and converted into AFM1, which is classified as a probable human carcinogen (Group 2B) by the International Agency for Research on Cancer (Karaoğlan et al., 2022; Mortaş et al., 2022).

Due to its harmful effects, particularly on children, the presence of AFM1 in food is regulated by food safety standards. The Turkish Food Codex Contaminants Regulation limits AFM1 levels to 0.05 µg/kg in raw milk, heat-treated milk, and milk used for dairy production, and 0.025 µg/kg in infant formulas (Turkish Food Codex, December 29, 2011, Issue: 28157). Cheese, a concentrated food product, can contain AFM1 levels 3-4 times higher than the milk used in its production (Mortaş et al., 2022).

Labneh cheese, originating from the Middle East and Egypt, is traditionally made by straining yogurt made from buffalo milk or a buffalo-cow milk mixture. This process involves salting the yogurt, straining it in cheesecloth for 12-24 hours, and storing the resulting smooth, creamy, acidic, and milk-white cheese under refrigeration (Abou-Donia, 2008). Studies on this subject have shown that labneh cheese can be produced by adding yoghurt cultures, lowering the pH, adding salt and rapidly stirring the mixture before heat treating it at 85°C for five minutes and hot filling it into containers (Sönmez, 2019).

This study aimed to investigate the AFM1 levels in labneh cheese samples available in Afyonkarahisar using the Enzyme-Linked Immunosorbent Assay (ELISA) method, identifying potential public health risks.

MATERIAL and METHOD

Study Material

A total of 40 randomise labneh cheese samples, representing 20 different brands and two batch numbers per brand, were collected from retail outlets in Afyonkarahisar between July and September 2023. Samples were transported to the laboratory under cold chain conditions.

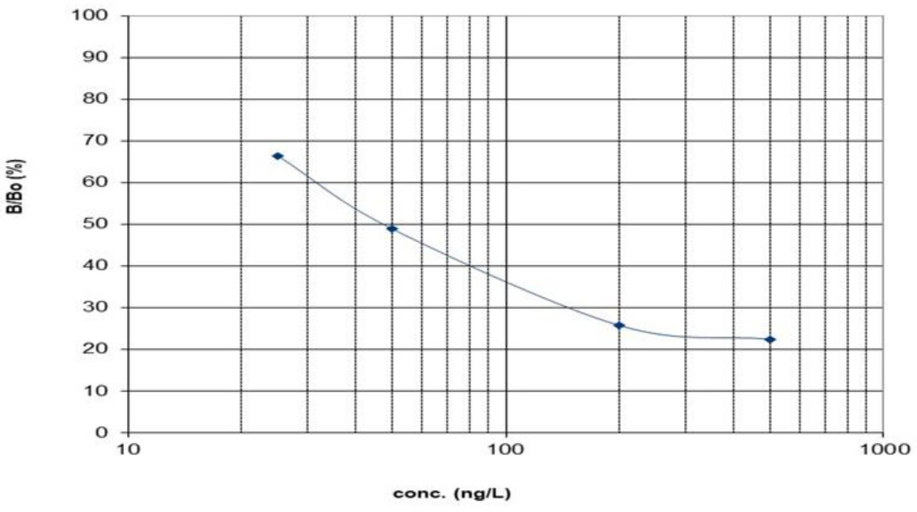
Sample Preparation

One gram of cheese was homogenized with 4 mL of 70% methanol and centrifuged at 10°C, 3000 rpm, for 10 minutes. Following centrifugation, 0.4 mL of the fat-free aqueous phase was transferred into sterile 15 mL containers, mixed with 0.4 mL of hexane, and vortexed for 10 seconds. The mixture was centrifuged again under the same conditions. The aqueous phase was diluted at a 1:5 ratio, and 100 µL of the final solution was used for analysis (Akgül and Kara, 2021; Aksoy and Sezer, 2019).

ELISA for Aflatoxin M1 Detection

AFM1 levels in the collected labneh cheese samples were measured using an ELISA reader (Thermo MultiScan) and a commercially available ELISA kit (AflaM1, Celer AFLA M1 500; detection limit: 25 ng/L). Absorbance values obtained from standards with known concentrations (0, 25, 50, 200, and 500 ng/L) were used to calculate results. The standard curve for aflatoxin concentrations is shown in Figure. Before use, ELISA kits and reagents were equilibrated to room temperature. Standards and extracted sample solutions (100 µL each) were added to the respective wells as per the manufacturer's instructions. The ELISA plate was covered with a transparent film, manually shaken for 5 seconds, and incubated at room temperature for 10 minutes. After discarding the liquid from the wells, they were washed three times with washing buffer. Subsequently, 100 µL of enzyme conjugate solution was added to each well, covered with a transparent film, and incubated for 5 minutes at room temperature. The washing step was repeated, and all wells were filled with the substrate solution using a multichannel micropipette. The plate was covered with a transparent film, gently shaken for 5 seconds, and incubated for 5 minutes to allow colour development. Finally, 50 µL of stop solution was added to each well, and absorbance values were measured at 450 nm.

Figure. Standard curve for Aflatoxin M1 concentration.



RESULTS

Analysis of the 40 labneh cheese samples revealed that AFM1 was detected in four samples (10%), with concentrations ranging from 0.026 to 0.033 µg/kg. All samples were within the AFM1 limit set by the Turkish Food Codex Contaminants Regulation (0.050 µg/kg) (Table 1). Other samples were below the detection limit.

Table 1. AFM1 Levels in Positive Samples

Samples	AFM1 Levels	
	ng/kg	**µg /kg**
1	26,347	0,026
2	27,225	0,027
3	32,475	0,032
4	32,709	0,033

N:40

DISCUSSION

The presence of AFM1 in milk and dairy products is a significant concern, particularly in developing countries (Iqbal et al., 2015). Manetta et al. (2009) reported a direct relationship between the level of AFM1 in milk and its concentration in final dairy products. Anfossi et al. (2012) found that Italian cheeses made from goat's and sheep's milk were less contaminated with AFM1 than those made from cows' milk. Cheese is a potential source of AFM1 due to its association with the casein fraction of milk. Industrial producers typically use a greater variety of milk sources, thereby reducing the likelihood of high AFM1 contamination. In contrast, small dairies often rely on a limited number of sources, which may contribute to higher contamination (Anfossi et al., 2012). Furthermore, labneh cheese, which is generally produced industrially from various milk sources, is expected to pose a lower risk of AFM1 contamination; however, its AFM1 level can be up to three times higher than that of the milk from which it is made (Ardıç et al., 2009). AFM1 levels are minimally affected by heat treatments (e.g., pasteurization, thermization), refrigeration, freezing, sterilization, or fermentation processes used in dairy technology (Prandini et al., 2009). Seasonal temperature variations and improper feed storage conditions influence aflatoxin levels in feed. AFM1 can be detected in milk as early as 12 hours after ingestion of AFB1-contaminated feed. Due to its strong binding to casein, AFM1 is found at concentrations 3-7 times higher in dairy products than in milk, posing severe health risks (Toptaş and Erköse Genç, 2023; Erol, 2022). Consumption of AFM1-contaminated dairy products by lactating women has also been reported to pose risks to infants (Benkerroum and Amir, 2022).

Studies conducted in Turkey on AFM1 levels in cheese and other dairy products have reported numerous cases exceeding and falling below regulatory limits. For example, Mortaş et al. (2022) found that 1.2% of 83 cheese samples from Ankara contained AFM1 levels above the Turkish Food Codex limits. Similarly, Aksoy and Sezer (2019) reported measurable AFM1 levels in 60 out of 150 cheese samples, although none exceeded the legal limit. - However, in a study using ELISA to analyze 193 cheese samples from Erzurum, 26.4% exceeded the Turkish Food Codex limits (Ardıç et al., 2009). A study on 90 cheese samples from Diyarbakir revealed that 14.4% contained AFM1 levels above the limits (Erkan et al., 2009). Yaroğlu et al. (2005) investigated the levels of AFM1 in 600 cheese samples (200 Turkish white cheeses, 200 kashar cheeses and 200 cream cheeses) collected from various regions of Türkiye. AFM1 was detected in 5% of samples (30 in total), with an incidence rate of 5% for Turkish white cheese, 6% for kashar cheese and 4% for cream cheese. Variations in AFM1 levels between studies may result from differences in animal feeding practices, feed storage methods, and seasonal factors affecting aflatoxin levels (Karaoğlan et al., 2022). Studies and results on AFM1 levels in cheeses are shown in Table 2.

Table 2. Studies and results on AFM1 levels in cheeses

Source	Product	Samples (N)	Positivity	AFM1 Levels
Ardıç et al. (2009)	Turkish White Cheese	193	82.4% (159)	52–860 ng/kg (51 samples > 250 ng/kg)
Gücükoğlu et al. (2010)	Various local cheeses	64	-	11 samples above legal limit
Turgay et al. (2010)	Semi-Hard Cheeses	Total 46; (22 cows, 18 goats, 6 sheep)	Sheep 0%, Cow and Goat 80%	Cow: 0.06–1.20 ng/g, Goat: 0.06–0.22 ng/g
İşleyici et al. (2011)	Divle Tulum Cheese	55	18.18% (10)	5.15–26.44 ng/kg (Average: 10.83 ± 6.7)
Dinçel et al. (2012)	Civil, Mihalıç, Kars Kashar, Otlu, Urfa Cheeses	100 (20 each)	Urfa 50% (10)	Average 0.036 µg/kg
Rubio et al. (2011)	Curd, Manchego Cheese, Whey	-	-	Curd 2-2.7x, After Ripening 2.7-2.9x (139.0–221.3 ng/kg)
Manetta et al. (2009)	Grana Padano Cheese and Curd	-	-	Grana Padano 4.5x, Curd 3x higher

CONCLUSION

Studies on AFM1 levels in milk and dairy products have shown a wide range of concentrations. A review of previous studies on AFM1 levels in cheeses worldwide reveals a consistent trend of higher levels in developing and least developed countries with temperate or tropical climates, particularly during warm seasons. This phenomenon may be attributed to variations in feed storage conditions. It is hypothesised that industrial cheeses, which are manufactured using milk sourced from multiple producers, contain lower levels of AFM1 than local cheeses made from milk provided by a limited number of producers. Given that labneh cheeses in Turkey are typically produced on an industrial scale, a positivity rate of 10% for AFM1 is considered to be high. In this study, AFM1 was detected in only four of the 40 labneh cheese samples, all within the Turkish Food Codex limits. Considering the potential public health risks associated with AFM1, preventive measures should be implemented throughout the food chain, from raw material production to final consumer delivery. Strict hygiene regulations and penalties for non-compliance, along with producer education, are crucial to reducing AFM1 levels.

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Ethics Approval

Ethics committee decision is not required.

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Conflict of Interest

There is no conflict of interest in the study.

Author Contributions

Research Design (CRediT 1) Author 1 (%40) – Author 2 (%20) – Author 3 (%20) – Author 4 (%20)
Data Collection (CRediT 2) Author 1 (%20) – Author 2 (%20) – Author 3 (%30) – Author 4 (%30)
Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%30) – Author 2 (%30) – Author 3 (%20) – Author 4 (%20)
Writing the Article (CRediT 12-13) Author 1 (%30) – Author 2 (%20) – Author 3 (%20) – Author 4 (%20)
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Sustainable Development Goals (SDG)

2 Zero Hunger
3 Good Health and Well-Being

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